

AMENDMENT

IN THE SPECIFICATION

Please amend the specification as follows:

At page 1, line 1, before "INSECT-RESISTANT PLANTS", please insert a new paragraph (subheading) --Title of the Invention--.

At page 1, line 8, before "*Bacillus thuringiensis* (*B.t.*) is a spore", please insert a new paragraph (subheading) --Background of the Invention--.

At page 2, line 10, before "Although certain chimeric", please insert a new paragraph (subheading) --Brief Summary of the Invention--.

At page 4, please replace paragraphs 2-8, corresponding to lines 9-26, with the following replacement paragraphs:

--Figure 1 shows the DNA probes (SEQ ID NOS:4-8) used for isolation of the *B.t.t.* toxin gene.

Figure 2 shows the steps employed in the preparation of plasmid pMON5432.

Figure 3 shows the orientation of the 3.0 kb HindIII fragment encoding the toxin gene in pMON5420 and pMON5421 with respect to the multilinker of pUC119.

Figure. 4 shows the strategy utilized for sequencing of the *B.t.t.* toxin gene contained in pMON5420 and pMON5421.

Figure 5 shows the DNA sequence and location of restriction sites for the 1932 bp ORF (SEQ ID NO:1) of the *B.t.t.* gene encoding the 644 amino acid toxin protein (SEQ ID NO:2).

Figure 6 shows the bands observed for *B.t.t.* toxin following SDS-PAGE analysis.

Figure 7 shows the N-termini of proteins expressed from the *B.t.t.* toxin gene or proteolytically produced *in vivo* in *B.t.t.* (SEQ ID NO:2, amino acids 1-300).--

At page 5, please replace paragraph 1, corresponding to lines 1-2, with the following replacement paragraph:

-- Figure 10 shows the deletions produced in evaluation of *B.t.t.* toxin protein mutants (SEQ ID NO:2). --

At page 5, please replace paragraph 9, corresponding to lines 18-19, with the following replacement paragraph:

-- Figure 18 shows the DNA sequence for the enhanced CaMV35S promoter (SEQ ID NO:33).--

At page 9, please replace paragraph 3, corresponding to lines 27-34, with the following replacement paragraph:

-- All protein structures represented in the present specification and claims are shown in conventional format wherein the amino group at the N-terminus appears to the left and the carboxyl group at the C-terminus at the right. Likewise, amino acid nomenclature for the naturally occurring amino acids found in protein is as follows: alanine (Ala; A), asparagine (Asn;N), aspartic acid (Asp;D), arginine (Arg;R), cysteine (Cys;C), glutamic acid (Glu;E), glutamine (Gln;Q), glycine (Gly;G), histidine (His;H), isoleucine (Ile;I), leucine (Leu;L), lysine (Lys;K), methionine (Met;M), phenylalanine (Phe;F), proline (Pro;P), serine (Ser;S), threonine (Thr;T), tryptophan (Trp;W), tyrosine (Tyr;Y) and valine (Val;V).--

At page 12, please replace paragraph 1, corresponding to lines 1-9, with the following replacement paragraph:

--Peak A and B (SEQ ID NO:3, amino acids 1-15):

1	5	10	15
Met	Asn	Pro	Asn
Asn	Arg	Ser	Glu
His	Asp	Thr	Ile
Lys	Thr	Thr	

Peak C (SEQ ID NO:34):

1	5	10	15
Met	X	Pro	X
Thr	Arg	Ala	Leu
Asp	Asp	Thr	Ile
Lys	Lys	Asp	

X represents an undeterminent amino acid.--

At page 23, please replace Table 3, corresponding to lines 17-30, with the following replacement Table 3:

-- **TABLE III**

Synthetic Oligonucleotides Used for Sequencing the <i>B.t.t.</i> Insecticidal Toxin Gene			
<u>Primer</u>	<u>Template</u>	<u>Sequence</u>	<u>Location</u> ¹
Bttstart (SEQ ID NO:35)	pMON5420	tgaacatggtagttgg	291-275
Bttext (SEQ ID NO:36)	pMON5421	taggtgatctctaggcg	422-439
Bttseq (SEQ ID NO:37)	pMON5421	ggaacaaccttctctaata	1156-1175
BttA1* (SEQ ID NO:38)	pMON5421	atgaayccnaayaaycg	205-222
BttA2* (SEQ ID NO:39)	pMON5421	garcaaygayacyathaa	227-242

* y = t or c. r = a or g. h = t, c or a. n = a, g, c or t.

¹ The location of the primers is based on the total of 2615 bases sequenced. Sequencing from pMON5420 proceeded toward the amino acid end and from pMON5421 toward the carboxyl end (see Figure 3). --

At page 29, please replace paragraph 2, corresponding to lines 12-32, with the following replacement paragraph:

-- Desired Site Primer (SEQ ID NO:40)

NcoI GATTGTTTCGGATCCATGGTTCTTCCTCCCT

The generation of the NcoI site at the N-terminus has changed the second amino acid from asparagine to aspartic acid. This change does not affect insect toxicity. BamHI and StyI sites have also been generated as a consequence of the introduction of this NcoI site. The plasmid containing the NcoI site has been designated pMON9759. The 2.5 kb NcoI-HindIII fragment containing the toxin encoding segment from pMON9759 was then cloned into NcoI-HindIII digested pMON5634 to produce pMON5436. Referring to Figure 16, pMON5634 is a pBR327 based plasmid which also contains the f1 phage origin of replication. The vector contains a synthetic *recA* promoter which is induced by nalidixic acid. The gene 10 leader from phage T7 (described in --

At page 38, please replace paragraph 1, corresponding to lines 3-18 with the following replacement paragraph:

--Construction of pMON5438 (HpaI, C-terminal Deletion of 463 bp)

pMON5420 was digested with HpaI and ligated with the following synthetic terminator linker. The linker contains nonsense codons in each reading frame and a BglII 5' overhang.

5'-TAGTAGGTAGCTAGCCA-3' (SEQ ID NO:41)
3'-ATCATCCATCGATCGGTCTAG-5' (SEQ ID NO:42)

The ligation was digested with BglII, to remove multiple linker inserts and then re-ligated. The ligation was transformed into JM101 and pMON5430 was isolated. To generate a NcoI site at the start of the truncated gene, the 2.32 kb PstI fragment of pMON9759 was replaced with the 1.47 kb PstI fragment of pMON5430 and the new construct was designated pMON5434. The 1.57 kb NcoI/HindIII fragment from pMON5434 was cloned into the *E. coli* high expression vector pMON5634, to create pMON5438. --

At page 40, please replace paragraph 2, corresponding to lines 17-31, with the following replacement paragraph:

-- Construction of pMON5456 (Band 3 Mutant, N-terminal Deletion of 140 bp)

A NcoI site was introduced into pMON5420 at the ATG for band 3 by site directed mutagenesis as described above using the primer:

Mutagenesis Primer – BTTLOOP (SEQ ID NO:43)
CGTATTATTATCTGCATCCATGGTTCTTCCTCCCT

to create pMON5455. The mutagenesis also deleted the upstream sequence which encodes the N-terminal 48 amino acids of band 1. The NcoI/HindIII fragment from pMON5455 was cloned into the *E. coli* high expression vector pMON5634 to create pMON5456. This plasmid expresses only band 3. The generation of the NcoI site changes the second amino acid from threonine to aspartic acid. --

At page 41, please replace paragraphs 1-2, corresponding to lines 1-25 with the following replacement paragraphs:

-- Construction of pMON5460 (Mutant Band 1 Gene with MET48 Changed to ILE)

The codon for methionine at position 48 in pMON9759 was changed to a codon for isoleucine by site directed mutagenesis as described above using the primer:

Mutagenesis Primer - BTTMET (SEQ ID NO:44)

ATTATTATCTGCAGTTATTCTTAAAACTCTTTAT

to create pMON5458. The NcoI/HindIII fragment of pMON5458 was cloned into the *E. coli* high expression vector pMON5634 to create pMON5460. By removing the ATG codon which initiates translation of band 3 protein, pMON5460 produces only band 1 protein with an isoleucine residue at position 48.

Construction of pMON5467 (Band 5 Mutant, N-terminal Deletion of 293 bp)

A NcoI site was introduced into pMON5420 to create a N-terminal deletion of ninety-eight amino acids by site directed mutagenesis using the primer:

Mutagenesis Primer (SEQ ID NO:45)

TCACTTGGCCAAATTGCCATGGTATTATAAAAAGTTTGT

to create pMON5466. A methionine and alanine were also inserted by the mutagenesis. The NcoI/HindIII fragment from pMON5466 was cloned into the *E. coli* high expression vector pMON5634 to create pMON5467.--

At page 44, please replace paragraph 1, corresponding to lines 1-15, with the following replacement paragraphs:

--CONSTRUCTION OF PLANT TRANSFORMATION VECTORS

The *B. t. var. tenebrionis* toxin gene contained in pMON5420 was modified for incorporation into plant expression vectors. A BglII site was introduced just upstream of the ATG codon which specifies the initiation of translation of the full-length *B.t.t.* toxin protein

(referred to as band 1) using the site specific mutagenesis protocol of Kunkel (1985) as previously described. The sequence of the *B.t.t.* toxin gene in the region of the initiator ATG is:

ATGATAAGAAAGGGAGGAAGAAAAATGAATCCGAACAATCGAAGTGAACATGATACAATA (SEQ ID NO:46)

MetAsnProAsnAsnArgSerGluHisAspThrIle (SEQ ID NO:47)

The primer for this mutagenesis (bttbgl) was 27 nucleotides in length and has the sequence:

CGGATTCATT TTAGATCTTC CTCCTT (SEQ ID NO:48)--

At page 47, please replace paragraph 1, corresponding to lines 1-21, with the following replacement paragraph:

-- pMON9753 contained approximately 400 bp of 3' noncoding sequence beyond the termination codon. Since this region is not necessary for toxin production it was removed from the *B.t.t.* toxin gene segments inserted in pMON893. In order to create a *B.t.t.* toxin gene containing no 3' flanking sequence, a BglII site was introduced just after the termination codon by the method of Kunkel (1985). The sequence of the *B.t.t.* toxin gene around the termination codon is:

GTTTATATAGACAAAATTGAATTTATTCCAGTGAATTAAATTAAGTAAAGAAG (SEQ ID NO:49)

ValTyrIleAspLysIleGluPheIleProValAsnEnd (SEQ ID NO:50)

Mutagenesis was performed with a primer (bttcterm) of sequence:

CTTTCTAGTT AAAGATCTTT AATTCAGT (SEQ ID NO:51)

Mutagenesis of the *B.t.t.* toxin gene was performed in pMON9758. A plasmid which contains the new BglII site was designated pMON9787 (Figure 12). Because pMON9787 contains a BglII site just upstream of the ATG initiation codon, the full coding sequence for the *B.t.t.* toxin gene with essentially no 5' or 3' flanking sequence is contained on a BglII fragment of about 1940 bp.--

At page 48, please replace paragraph 1, corresponding to lines 1-12, with the following replacement paragraph:

-- that, as was the case for the *B.t.k.* gene, truncated forms of the *B.t.t.* gene might be more easily expressed in plant cells. Therefore, a modified *B.t.t.* toxin gene was constructed in which the region upstream of the band 3 ATG codon has been removed. In order to remove this sequence, a BglII site was inserted just upstream of the band 3 ATG by the method of Kunkel (1985). The sequence surrounding the band 3 ATG is:

CCAAATCCAACACTAGAAGATTTAAATTATAAAGAGTTTTTAAGAATGACTGCAGATAAT (SEQ ID NO:52)

ProAsnProThrLeuGluAspLeuAsnTyrLysGluPheLeuArgMetThrAlaAspAsn (SEQIDNO:53)

Mutagenesis was performed with primer (bttnterm) of sequence:

ATCTGCAGTC ATTGTAGATC TCTCTTTATA ATTT (SEQ ID NO:54)--